Respiratory Medicine (2007) **101**, 696–705

ELSEVIER

respiratoryMEDICINE

REVIEW

Eosinophil cationic protein: Is it useful in asthma? A systematic review ☆

Gerald C.-H. Koh[a,*], Lynette P.-C. Shek[b], Daniel Y.-T. Goh[b], Hugo Van Bever[b], David S.-Q. Koh[a]

[a]*Department of Community, Occupational and Family Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Blk MD3, 16 Medical Drive, Singapore 117597, Singapore*
[b]*Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074, Singapore*

Received 23 March 2006; accepted 14 August 2006

KEYWORDS
Eosinophil cationic protein;
ECP;
Asthma;
Biological marker;
Systematic review

Summary
Introduction: Eosinophil cationic protein (ECP) has been widely investigated as a potential biomarker of airway inflammation.
Method: A systematic review was performed using Medline with key terms *eosinophil cationic protein* and *asthma*, limiting the search to titles or abstracts. Out of 688 potential papers found, abstracts were reviewed based on the following criteria: (1) ECP was used as a biological marker, (2) asthma was the index disease studied, (3) it was a controlled clinical study and (4) ECP was assessed as a diagnostic, assessment or management tool. One hundred and sixty-nine articles satisfied the selection criteria and their full-text versions were reviewed. Only 53 papers were found to provide clinically useful information.
Results: ECP has been measured in serum, plasma, sputum, saliva and broncho-alveolar lavage fluids but serum and sputum are the most established. Levels of ECP in normal and asthmatic subjects in various body fluids were identified. ECP correlates well with airway inflammation but not airway hyper-responsiveness. It is raised in other atopic diseases and hence is not diagnostic for asthma. However, it has been shown to be useful in assessing asthma severity, compliance with anti-inflammatory asthma therapy and as a guide to tailing down inhaled corticosteroid therapy. Although there is some evidence that ECP levels are affected by age, smoking, circadian rhythm and seasonal variation, only smoking appears to be of clinical significance.

☆Statement of Conflict of Interest: GCHK, LPCS, VBHPS and DSQK has no declared conflict of interest; DYTG is a member of the Pediatric Advisory Board for consumer product research at Johnson & Johnson since 2004 and has received funding for asthma research from Merck Sharpe & Dohme, Pfizer, Astra Zenec and Merck & Co., Inc.

*Corresponding author. Tel.: +65 65164979; fax: +65 67791489.
E-mail address: cofkohch@nus.edu.sg (G.C.-H. Koh).

0954-6111/$ - see front matter
doi:10.1016/j.rmed.2006.08.012

Discussion: Despite its limitations, ECP remains potentially useful in asthma management. Future research on ECP should focus on using serial measurements and combining it with other markers of asthma which may increase its clinical usefulness.

Contents

Introduction

To date, the assessment of asthma has been based mainly on surrogate measures of airway inflammation such as airflow limitation or reversibility using spirometry and related techniques. Direct measurement of airway inflammation using biological markers could potentially refine asthma management and this explains current research interest in exhaled nitric oxide and eosinophil granule proteins in asthma.[1] In recent years, clinical research has suggested an emerging clinical usefulness of eosinophil granule proteins in the assessment and management of asthma, of which eosinophil cationic protein (ECP) has been most widely characterized and researched. The aim of this paper is to systematically review the current evidence for the usefulness on ECP in asthma management and suggest future areas for clinical research.

Methods

A systematic review was performed using the Quality of Reporting of Meta-Analysis (QUORUM) Guidelines.[2] A Medline search using the key terms *eosinophil cationic protein* and *asthma* was performed on 2 January 06, limiting the search to title or abstract of papers and to humans only. A similar search in major respiratory journals (i.e. Thorax, American Journal of Respiratory and Critical Care Medicine, European Respiratory Journal, Respiratory Medicine and Journal of Asthma) was also performed. Six hundred and eighty-eight potential papers were found. The abstracts were then reviewed based the following selection criteria: (1) ECP was used as a biological marker (regardless of body fluid sampled), (2) asthma was the index disease studied (regardless of type of asthma), (3) it was a controlled clinical study, (preferably but not limited to randomized controlled trials) and (4) ECP was assessed as a diagnostic, assessment or management tool. Only 169 articles potentially satisfied the selection criteria. Full-text versions of the papers were then obtained and reviewed. Relevant references cited in the papers but not identified by the Medline search were also obtained and reviewed. After full-text review, only 53 papers were found to fulfill the selection criteria. A flow chart detailing the systematic review process is found in Fig. 1. The results of the review are divided into two sections: the first part gives an overview of the biological properties and issues of sampling ECP from various body fluids and the second part reports the uses and limitations of ECP in asthma management.

Results

Properties, characteristics and genetics of ECP

ECP is one of the four major cationic granule proteins released by activated eosinophils [the other three being

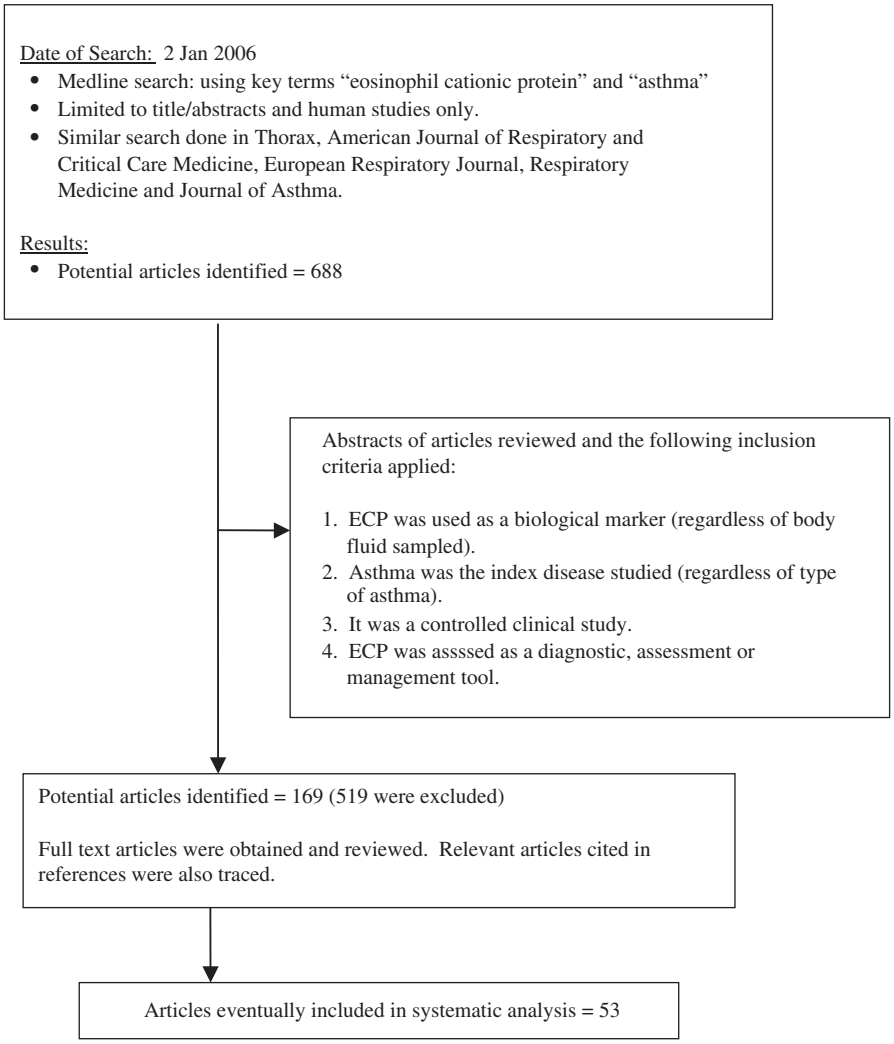

Figure 1 Stages of systematic analysis.

eosinophil protein X (EPX), eosinophil peroxidase (EPO) and major basic protein (MBP)] and is presently the most widely used clinical marker of eosinophil activity in asthma.[3] ECP is a single-chain, zinc-containing protein with a molecular weight ranging from 16 to 22 kDa. The heterogeneity of the molecule is partially due to differences in glycosylation of three potential sites in its amino acid chain. The mechanism of action of ECP is due to its cytotoxic capacity to create pores in cell membranes.[4] It has been shown to have cytotoxic effects against bacteria, parasites, viruses and respiratory epithelial cells.[5–7] ECP also stimulates mucous production in airways and histamine release by basophils and mast cells in vitro.[8–10] ECP is synthesized in eosinophil progenitors in human bone marrow and stored in specific granules in mature peripheral blood eosinophils. Triggers of eosinophil release of ECP include immunoglobulin G complexes and interleukin-5. *In vitro*, corticosteroids at pharmacological doses appear to be unable to inhibit ECP release from eosinophils. A more detailed account on the properties and characteristics on ECP can be found in a paper by Venge et al.[11] The gene that codes for ECP has been located on chromosome 14q11.2 and three polymorphisms have been identified by Noguchi et al.[12] Although, no relationship between ECP gene polymorphisms and asthma was found in this study, serum ECP levels of persons with the −393T allele was lower than those with the −393C allele. This may explain why serum ECP in some asthmatic patients is not raised and the authors suggested that ECP measurement in future asthma studies should include with genotyping for −393C/T polymorphism.

ECP in body fluids

ECP can be measured in many different body fluids such as serum, plasma, sputum, saliva, nasal lavage fluid, bronchoalveolar lavage fluid and urine.[3,11,13] The levels (normal and asthmatic) and advantages and disadvantages of ECP measurement in various body fluids are detailed in Table 1.

Table 1 Levels (normal and asthmatic), advantages and disadvantages of ECP measurement in various body fluids.

Body fluid	Range of ECP levels (μg/L)		Advantages	Disadvantages
	Normal	Asthmatics		
Serum[11,13,14]	2–20	>20	Reflects propensity of activated eosinophils to release granule proteins	Blood must be collected under tightly controlled and standardized conditions
Plasma[11,14]	5–10 times lower than in serum		Reflects true levels of ECP in blood	Low levels are found because EDTA inactivates *ex-vivo* release of ECP
Sputum[15–17]	20–1280	76.8–32,000	High levels of ECP found	• Requires induction of sputum with nebulized saline in non-productive asthmatics • There is wide overlap in range of normal and asthmatics
Saliva[17]	250–450	450–850	• High levels of ECP found • Easy and painless collection process	Insufficient research to support its repeatability and clinical utility
Nasal lavage fluid[20–23]	0–60	90–120*	Painless but uncomfortable collection process	• Difficult to obtain samples • Does not correlate well with asthma
Broncho-alveolar lavage fluid[24,25]	1–3	5–100	Moderate levels of ECP found	Difficult to obtain samples
Urine[3,13,20]	—	—	—	Not useful. Urine eosinophil protein X (EPX)/urine creatinine ratio is more useful. However, urinary EPX is not widely used in asthma research

*Expressed as μg of ECP per g of protein.[21]

Serum and plasma ECP

The levels of ECP in serum is often five to ten times of those in plasma because eosinophils in blood containing EDTA are inactivated and do not continue their extracellular release of ECP *ex vivo*.[14] Hence, serum ECP levels reflect circulating ECP and the propensity of active eosinophils to release ECP ex vivo, which may explain why the clinical information obtained using serum ECP have been found to be more clinically useful than plasma ECP.[11] ECP secretion in blood samples can also increase by two to four times at 37 °C relative to 22 °C when stored for an hour.[14] Therefore, serum sampling of ECP should be standardized as far as possible because levels can vary depending on the storage time and ambient temperature. The serum ECP level for both non-atopic adults and children is less than 20 μg/L.[13]

Sputum ECP

ECP is also present in the sputum. The median range of ECP in sputum of non-asthmatics is about 200–350 μg/L, which is higher than serum levels. A major challenge with sputum measurement of ECP is that it has to be induced in non-productive subjects with nebulized saline. Immediate mucous and cell dispersion and cationic detergent addition are needed for sputum to avoid ECP destruction or variable attachment to test-tube walls and mucus. There is wide overlap between reported values of ECP in normal subjects which is likely due to the different reported methods of analysis.[15,16] Standardization of sputum ECP analysis is needed before it is used more widely and important aspects of this issue are well discussed in a recent paper by a sputum analysis working group led by Kelly et al.[17]

Salivary ECP

ECP has also been measured in saliva in adults and has been found to correlate with asthma severity.[18] Saliva ECP ranges from 250 to 500 μg/L and levels were associated with presumed activity of disease as recorded by alteration of taken dose of inhaled corticosteroids (ICS). The reason why

levels of ECP in sputum and saliva are 10 times higher than serum is unclear. However, Schmekel et al.[18] postulated that sputum and saliva ECP reflected a pathophysiological consequence of eosinophils present in the whole extent of the airways and oral mucosa. Another possible reason is that circulating eosinophils in blood retain their granule contents until they reach diseased tissue, a hypothesis supported by a recent study by Malm-Erjefalt et al.[19] The measurement of ECP in saliva is not well established and needs standardization before it becomes an accepted biological medium of assaying ECP in asthmatics.

ECP in nasal and broncho-alveolar fluid

ECP can also be found in nasal[20–23] and broncho-alveolar lavage fluids.[24,25] However, the collection of these fluids by nasal and broncho-alveolar lavage is highly invasive and unlikely to enter mainstream clinical use. Moreover, the measurement of ECP in nasal and broncho-alveolar fluid is not well standardized in terms of dilution frequency and internal standards.[24] Nasal lavage ECP was previously found to poorly correlate with asthma severity, although a recent study showed nasal lavage ECP correlated with spirometric parameters.[20,21]

ECP in urine

Urinary ECP has found to be inferior to urinary EPX [also known as eosinophil-derived neurotoxin (EDN)] as a marker of eosinophil turnover and activity in asthma.[3,13,20] Hence, ECP in urine is not useful as a marker of asthma severity.

Clinical significance of ECP in asthma

ECP and airway inflammation

Numerous studies have shown that ECP correlates well with airway inflammation in asthma. Serum ECP was found to directly correlate with activated eosinophils in bronchial mucosa biopsied from adult asthmatic patients.[26] Serum ECP has also been found to be a more sensitive marker of asthma severity than peripheral blood eosinophil counts in acute exacerbations in children. In this study that compared serum ECP and peripheral blood eosinophil count in 46 asthmatic children, the ratio of ECP to eosinophil count was significantly greater during acute exacerbation than during clinical remission [0.104 ± 0.049 versus 0.84 ± 0.041 (μg/L), $P < 0.05$].[27] The authors hypothesized that eosinophils released ECP preferentially during acute exacerbations than during clinical remission.

Sputum ECP has been found to be better than sputum eosinophils in characterizing oral corticosteroid dependent asthmatics with recent exacerbations.[28] In a study that compared serum ECP and sputum ECP with asthma severity using symptom scoring, sputum eosinophils and spirometry (FEV_1 and FEV_1/VC), sputum ECP was found to be a more sensitive marker of airway inflammation and asthma severity than serum ECP.[15]

Serum ECP rises during asthma exacerbations caused by upper respiratory infections.[29] However, a study between adult asthmatics experiencing exacerbations caused by para-influenza virus 3 (PIV3) and non-PIV3-induced exacerbations found no significant difference in their sputum ECP levels.[30] Another study found that serum ECP was raised in children with asthma but not in infants with respiratory syncytial virus (RSV) bronchiolitis.[31] These studies suggest that ECP levels are raised in virus-induced airway inflammation in asthma but are not affected by the type of virus triggering asthma nor raised in bronchiolitis.

ECP and airway hyper-responsiveness

Although ECP correlates with airway inflammation, studies in primates have found no correlation between ECP and airway hyper-responsiveness.[32] In a population-based study on atopy of 1189 persons in Denmark, there was no association between serum ECP and bronchial hyperreactivity as measured with methacholine challenge tests.[33] Similar findings have been found in other studies on asthma.[15,34–37] The reason is probably because ECP is not significantly related to the release of bronchoactive chemical mediators that increase the responsiveness of airway smooth muscle. In contrast, MBP which is another major cationic protein of eosinophil granules, has been found to induce airway constriction and hyper-responsiveness in primates.[32]

ECP as an asthma marker

ECP as a diagnostic tool

There is strong evidence that sputum ECP is raised in asthmatics, both in children and adults, and is increased in classic asthma, cough-variant asthma and occupational asthma.[35,36,38–40] However, ECP is also raised in other atopic diseases like allergic rhinitis and in pre-school children with recurrent wheezing.[41–43] ECP is also elevated in conditions that are not associated with eosinophil inflammation or atopy. For example, ECP in nasal lavage fluid is also associated with rhinovirus infections and bacterial sinusitis.[22,44] Hence, ECP is not a useful diagnostic marker for asthma because of its poor specificity.[45] It is worth noting that serum ECP is poorly associated with exercise-induced asthma, atopic eczema and food allergies.[46–48] In allergic rhinitis, nasal lavage ECP has been found to increase with nasal allergen challenge [23] and seasonal allergen exposure [41] but reduced by immunotherapy.[42,49]

ECP as an assessment tool

There is now a good body of evidence that ECP can be used to assess asthma severity. Serum ECP has been shown to correlate with severity of asthma as classified by STEP classification of the National, Heart, Lung and Blood Institute (NHLBI),[50] FEV_1,[50] FEV_1/FVC,[50] peripheral blood eosinophil count[27] and severity of atopy based on wheal size and number of positive prick tests.[36] Sputum ECP has also been found to correlate with asthma severity based on NHLBI [51] and Japanese Society of Allergology criteria,[16] PEFR,[16,51] sputum eosinophils,[16] need for oral corticosteroid therapy[28] and FEV_1 in treated asthmatics.[51] Although, ECP correlates with STEP classification of asthma severity, there is little difference in sputum ECP levels between mild persistent and moderate asthmatic patients attending an asthma clinic.[51] Hence, ECP is useful mainly in distinguishing between mild intermittent and severe asthmatics. This finding is interesting in the light of a 10-year longitudinal cohort study by de Marco et al. which found that patients with intermittent or mild persistent asthma were a different

phenotype from moderate and severe persistent asthma, with the latter group characterized by high IgE levels, persistent cough/mucus hypersecretion and early deterioration of lung function.[52] A recent paper reported that serum ECP was positively correlated to air-trapping in asthma as measured by quantitative high-resolution computed tomography (HRCT) which in turn, correlated with FEV_1/FVC.[53]

Serum ECP levels in an asthmatic child increases significantly during asthma exacerbation, from $27.4 \pm 11.5\,\mu g/L$ at clinical remission to $41.7 \pm 16.9\,\mu g/L$ during acute exacerbation.[27] Sputum ECP is also significantly increased in adult oral corticosteroid-dependent asthmatics who are uncontrolled and still experiencing acute exacerbations (mean $= 580\,\mu g/L$) compared to controlled oral corticosteroid-dependent asthmatics (mean $= 194\,\mu g/L$).[27]

ECP as a management tool

A study by Prehn et al.[54] using serum ECP to guide anti-inflammatory treatment of asthma in children demonstrated the usefulness of ECP in deciding when to tail down therapy. Using a clinical algorithm, they titrated the dose of anti-inflammatory drugs ICS based on serum ECP levels taken monthly in 21 children over 12 months. Patients with persistently raised ECP $> 30\,\mu g/L$ remained on high doses of ICS (budesonide $400\,\mu g$ bd), those with ECP between 15 and $30\,\mu g/L$ were given budesonide $200\,\mu g$ bd and those with ECP $< 15\,g/L$ received sodium cromoglycate only. Compliance was counter-checked by weighing the metered dose inhalers each month. During the study period, there was no asthma attacks, need for emergency room treatment, hospital admission or rescue treatment with oral steroid therapy in all three groups of patients. This study supports strongly the usefulness of ECP in guiding asthma therapy, considering that 45% of adult asthmatics relapse after asthma exacerbation within 8 weeks.[55] Prehn et al. also found that patients who were non-compliant to ICS medication exhibited an increase in serum ECP. ICS was more effective than sodium cromoglycate in reducing serum ECP, improving symptoms and lung function. Several other studies also support the close relationship between ECP and use of corticosteroids, suggesting that ECP can be used as a marker of compliance to oral and ICS therapy.[56–59] However, it should be noted that the suppressive effect of ICS on ECP is not sensitive enough to reflect small changes in doses.[16,56]

ECP correlates with other forms on anti-inflammatory agents besides ICS and mast cell stabilizers. Sputum ECP declines with leukotriene receptor antagonist (LTRA) therapy [60] but the correlation is weaker when compared with ICS therapy.[34,61,62] Anti-inflammatory therapy with tacrolimus in aspirin-induced asthma is associated with declines in sputum ECP as well.[63] Immunotherapy in asthmatic children is also associated with decline in sputum ECP levels.[64] However, ECP is not affected by H1 anti-histamines or β_2-agonists when given as add-on therapy to corticosteroid-treated asthmatics.[65,66] The clinical uses of ECP are summarized in Table 2.

Factors that affect ECP levels in asthmatics

Age

There is some suggestion that ECP appears to be more sensitive to changes in asthma severity in adolescents than in pre-pubertal children.[56] However, these findings need to be confirmed by a more rigorously designed study. A study by King et al.[67] suggested that serum ECP does not appear to be raised in elderly asthmatic patients, but his study was based on well community-living older persons and it did not measure the severity of asthma in its subjects.

Smoking

Sputum ECP in adult asthmatics who smoke is significantly higher than in non-smoking asthmatics.[51] Moreover, inhaled budesonide treatment reduces serum ECP and improves lung function in non-smoking asthmatics but not in smoking asthmatics.[68] This is expected as smoking is well known to cause chronic persistent inflammation of the airways. Hence, the smoking history of subjects should be considered when conducting ECP research or interpreting ECP results.

Circadian rhythm and seasonal variation

Wolthers and Heuck[69] reported circadian rhythm variations in serum ECP in children. They found that serum ECP levels reached a trough at 8.00 a.m. but climbed as the day progressed till the late evening and early morning, reaching a peak at 6.00 a.m. However, this circadian fluctuation is negligible when one considers that the range of ECP fluctuation in normal subjects was between 3.9 ± 0.7 and $16.3 \pm 3.5\,\mu g/L$ (mean $\pm$ SEM), and serum ECP in asthmatics is often raised above $20\,\mu g/L$. Serum ECP in non-atopic children aged 11–16 yr also exhibited seasonal variations with serum ECP decreasing from a median value of $14\,\mu g/L$ in May to $7\,\mu g/L$ in November.[70] These seasonal variations are probably due to seasonal environmental influences rather than daylight exposure, and the levels are so low and the range so narrow that it would be insignificant when serum ECP levels are used as an inflammatory marker of asthma.

ECP and other inflammatory markers of asthma

There are many inflammatory markers of asthma currently being investigated such as nitric oxide and their metabolites, arachidonic acid metabolites such as leukotriene E4 and 8-isoprostane, chemokines such as interleukins, γ-interferon, Th2-specific macrophage-derived chemokine and eosinophil chemoattractant, eotaxin. These markers are being measured with established methods such as exhaled breath, sputum and urine, as well as novel methods such as exhaled nasal air and exhaled breath condensate. There have been no published reports on head-to-head comparisons of these markers with ECP in asthma assessment or management, mainly because many of them are still in the early stages of research, with the exception of exhaled nitric oxide (eNO).[71] Yet, the measurement of eNO remains technically challenging, especially in children younger than 4 years, because it requires patient cooperation, strict standardized sampling techniques and a sustained minimal exhalation flow rate. On the other hand, saliva collection requires less patient cooperation and is independent of exhalation flow rate. Thus, further studies on the clinical utility of salivary ECP and its correlation with serum and sputum ECP are worthy endeavors. It is beyond the scope of this article to discuss other markers of airway

Table 2 Clinical significance of ECP in asthma.

Airway inflammation
- ECP correlates with activated eosinophils in bronchial mucosa of asthmatics[26]
- ECP is more sensitive than blood and sputum blood eosinophils in assessing asthma severity[27,28]
- Sputum ECP is more sensitive than serum ECP in assessing asthma severity[15]
- ECP levels appears to reflect virus-induced airway inflammation in asthma but is not affected by type of virus[29–31]

Airway hyper-responsiveness
- ECP does not correlate with airway hyper-responsiveness[15,33–37]

Diagnosis of asthma
- ECP is raised in other atopic diseases (e.g. allergic rhinitis, recurrent wheezing)[41–43] and infections (e.g. rhinovirus infections[22,44] and bacterial sinusitis[22])
- ECP is not useful as a diagnostic tool for asthma because of its lack of specificity

Assessment of asthma severity
ECP correlates with asthma severity when measured by
- NHLBI STEP classification[50,51]
- Japanese Society of Allergology criteria[16]
- PEFR[16,51]
- FEV_1[50,51]
- FEV_1/FVC[50]
- Peripheral blood eosinophil count[27]
- Sputum eosinophil count[16]
- Skin prick tests[36]

A guide to tailing down inhaled corticosteroid therapy[54]

Assessment of compliance
ECP decreases with treatment with anti-inflammatory asthma treatment such as
- Inhaled corticosteroids[54,56–59]
- Oral corticosteroids[57]
- Mast cell stabilizers[54]
- Leukotriene receptor antagonists[34,60–62]
- Tacrolimus[63]
- Immunotherapy[64]

inflammation measurement but we would like to direct the reader to relevant review articles for more details.[72,73]

Conclusions

ECP is found in many body fluids but serum and sputum sampling are the most established to date. It reflects airway inflammation and not hyper-responsiveness, and correlates with asthma severity as measured by symptom score and spirometry. As ECP is not specific to asthma, it is not useful as a diagnostic tool. However, it has shown to be useful in assessment of compliance to most forms of anti-inflammatory therapies in asthma and in guiding the tapering of ICS in stabilized asthmatics. The response of ECP levels, rather than the absolute value, to corticoste roid therapy in asthma is probably more useful in guiding management and predicting relapse after acute exacerbations.

The use ECP in asthma has its limitations. It has been argued that eosinophils are not the only cells involved in asthma, although it is recognized that they are key players in this inflammatory airway disease.[74] Not all forms of asthma have an atopic basis so the usefulness of ECP will be limited to allergic forms of asthma. Further research is needed before ECP can become a useful marker of airway inflammation to guide asthma therapy. For example, little is known about the relationship between eosinophils and ECP: Are the raised levels of ECP in asthmatics due to increased numbers of eosinophils, increased amounts of stored ECP within eosinophil granules or increased propensity of eosinophils to release ECP? We need to determine if ECP levels differ significantly between children, adults and elderly, and improve and standardize our collection methods to compare research results meaningfully. We also need to think beyond using ECP values as a single snapshot tool and begin using serial measurements to look at trends and see if they can predict asthma relapse and verify compliance and response to anti-inflammatory therapy. ECP may become even more useful if combined with other markers of asthma. ECP has the potential to meet our need for an airway inflammatory biomarker in asthma management but the key to its success lies in using what we currently know and translating it from bench to bedside.

Acknowledgments

This work was supported by Niche Area program Research Grant (Number: R-186-000-077-101) by the Yong Loo Lin School of Medicine, National University of Singapore. The authors wish to thank Andrew Wee and Vivian Ng for helping with collation of journal articles.

References

1. Lowhagen O, Wever AM, Lusuardi M, et al. The inflammatory marker serum eosinophil cationic protein (ECP) compared with PEF as a tool to decide inhaled corticosteroid dose in asthmatic patients. *Respir Med* 2002;**96**(2):95–101.
2. Moher DCD, Eastwood S, Olkin I, Rennie D. Stroup D for the QUORUM Group. Improving the qulaity of reports of meta-analyses of randomised controlled trials: the QUORUM statement. *Lancet* 1999;**354**:1869–900.
3. Venge P. Monitoring the allergic inflammation. *Allergy* 2004;**59**(1):26–32.
4. Young JD, Peterson CG, Venge P, Cohn ZA. Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* 1986;**321**(6070):613–6.
5. Lehrer RI, Szklarek D, Barton A, Ganz T, Hamann KJ, Gleich GJ. Antibacterial properties of eosinophil major basic protein and eosinophil cationic protein. *J Immunol* 1989;**142**(12):4428–34.
6. McLaren DJ, McKean JR, Olsson I, Venges P, Kay AB. Morphological studies on the killing of schistosomula of Schistosoma mansoni by human eosinophil and neutrophil cationic proteins in vitro. *Parasite Immunol* 1981;**3**(4):359–73.
7. Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF. Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. *Nucleic Acids Res* 1998;**26**(14):3358–63.
8. Hernnas J, Sarnstrand B, Lindroth P, Peterson CG, Venge P, Malmstrom A. Eosinophil cationic protein alters proteoglycan metabolism in human lung fibroblast cultures. *Eur J Cell Biol* 1992;**59**(2):352–63.
9. Bergstrand YHLB, Petersson B, Peterson CG, Venge P. Eosinophil cationic protein and human leukocyte histamine release. In: Venge PLA, editor. *Inflammation: basic mechanisms tissue inuring principles and clinical models*. Stockholm: Almqvist & Wiksell International; 1985. p. 361–5.
10. Patella V, de Crescenzo G, Marino I, et al. Eosinophil granule proteins are selective activators of human heart mast cells. *Int Arch Allergy Immunol* 1997;**113**(1–3):200–2.
11. Venge P, Bystrom J, Carlson M, et al. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy* 1999;**29**(9):1172–86.
12. Noguchi E, Iwama A, Takeda K, et al. The promoter polymorphism in the eosinophil cationic protein gene and its influence on the serum eosinophil cationic protein level. *Am J Respir Crit Care Med* 2003;**167**(2):180–4.
13. Koller DY. Sampling methods: urine/blood analysis. *Am J Respir Crit Care Med* 2000;**162**(2 Part 2):S31–3.
14. Bjork A, Venge P, Peterson CG. Measurements of ECP in serum and the impact of plasma coagulation. *Allergy* 2000;**55**(5): 442–8.
15. Pizzichini E, Pizzichini MM, Efthimiadis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. *J Allergy Clin Immunol* 1997;**99**(4):539–44.
16. Fujimoto K, Kubo K, Matsuzawa Y, Sekiguchi M. Eosinophil cationic protein levels in induced sputum correlate with the severity of bronchial asthma. *Chest* 1997;**112**(5):1241–7.
17. Kelly MM, Keatings V, Leigh R, Peterson C, Shute J, Venge P, et al. Analysis of fluid-phase mediators. *Eur Respir J* 2002;**37**: S24–39.
18. Schmekel B, Ahlner J, Malmstrom M, Venge P. Eosinophil cationic protein (ECP) in saliva: a new marker of disease activity in bronchial asthma. *Respir Med* 2001;**95**(8):670–5.
19. Malm-Erjefalt M, Greiff L, Ankerst J, et al. Circulating eosinophils in asthma, allergic rhinitis, and atopic dermatitis lack morphological signs of degranulation. *Clin Exp Allergy* 2005;**35**(10):1334–40.
20. Wojnarowski C, Roithner B, Koller DY, et al. Lack of relationship between eosinophil cationic protein and eosinophil protein X in nasal lavage and urine and the severity of childhood asthma in a 6-month follow-up study. *Clin Exp Allergy* 1999;**29**(7): 926–32.
21. Fal AM, Nowak AA, Nowak MT, Litwa M. Use of selected parameters of nasal lavage fluid in asthma severity monitoring. *Pneumonol Alergol Pol* 2004;**72**(9–10):375–82.
22. Niehaus MD, Gwaltney Jr. JM, Hendley JO, et al. Lactoferrin and eosinophilic cationic protein in nasal secretions of patients with experimental rhinovirus colds, natural colds, and presumed acute community-acquired bacterial sinusitis. *J Clin Microbiol* 2000;**38**(8):3100–2.
23. Pedroletti C, Lundahl J, Alving K, Hedlin G. Exhaled nitric oxide in asthmatic children and adolescents after nasal allergen challenge. *Pediatr Allergy Immunol* 2005;**16**(1):59–64.
24. Schmekel B, Hornblad Y, Hvatum M, Norlund AL, Venge P. Kinetic retrieval of eosinophil cationic protein, hyaluronan, secretory IgA, albumin, and urea during BAL in healthy subjects. *Chest* 1995;**108**(1):62–7.
25. Kim CK, Kim SW, Kim YK, et al. Bronchoalveolar lavage eosinophil cationic protein and interleukin-8 levels in acute asthma and acute bronchiolitis. *Clin Exp Allergy* 2005;**35** (5):591–7.
26. Hoshino M, Nakamura Y. Relationship between activated eosinophils of the bronchial mucosa and serum eosinophil cationic protein in atopic asthma. *Int Arch Allergy Immunol* 1997;**112**(1):59–64.
27. Koh YY, Kang H, Kim CK. Ratio of serum eosinophil cationic protein/blood eosinophil counts in children with asthma: comparison between acute exacerbation and clinical remission. *Allergy Asthma Proc* 2003;**24**(4):269–74.
28. Tarado De La Fuente PRM, Carlsson L, Godard P, Bousquet J, Chanez P. Eosinophilic inflammation assessed by induced sputum in corticosteroid-dependent asthma. *Respir Med* 1999; **93**(3):183–9.
29. Yasuda H, Suzuki T, Zayasu K, et al. Inflammatory and bronchospastic factors in asthma exacerbations caused by upper respiratory tract infections. *Tohoku J Exp Med* 2005;**207** (2):109–18.
30. Matsuse HKY, Saeki S, Nakata H, Fukushima C, Mizuta Y, Kohno S. Naturally occurring parainfluenza virus 3 infection in adults induces mild exacerbation of asthma ssociated with increased sputum concentrations of cysteinyl leukotrienes. *Int Arch Allergy Immunol* 2005;**138**(3):267–72.
31. Priftis KN, Papadopoulou A, Liatsis E, Katsikas D, Nicolaidou P, Kanariou M. Serum eosinophil cationic protein and CD23 in acute RSV bronchiolitis. *Med Sci Monit* 2005;**11**(10):CR493–7.
32. Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;**87**(4):1470–3.
33. Elberling J, Linneberg A, Dirksen A, et al. Mucosal symptoms elicited by fragrance products in a population-based sample in relation to atopy and bronchial hyper-reactivity. *Clin Exp Allergy* 2005;**35**(1):75–81.
34. Cakmak G, Demir T, Gemicioglu B, Aydemir A, Serdaroglu E, Donma O. The effects of add-on zafirlukast treatment to budesonide on bronchial hyperresponsiveness and serum levels

of eosinophilic cationic protein and total antioxidant capacity in asthmatic patients. *Tohoku J Exp Med* 2004;**204**(4):249–56.
35. Yoo Y, Koh YY, Kang H, Yu J, Nah KM, Kim CK. Sputum eosinophil counts and eosinophil cationic protein levels in cough-variant asthma and in classic asthma, and their relationships to airway hypersensitivity or maximal airway response to methacholine. *Allergy* 2004;**59**(10):1055–62.
36. Joseph-Bowen J, de Klerk N, Holt PG, Sly PD. Relationship of asthma, atopy, and bronchial responsiveness to serum eosinophil cationic proteins in early childhood. *J Allergy Clin Immunol* 2004;**114**(5):1040–5.
37. Liu SF, Lin MC, Chang HW. Relationship of allergic degree and PC20 level in adults with positive methacholine challenge test. *Respiration* 2005;**72**(6):612–6.
38. Konno S, Gonokami Y, Kurokawa M, et al. Cytokine concentrations in sputum of asthmatic patients. *Int Arch Allergy Immunol* 1996;**109**(1):73–8.
39. Dominguez Ortega JLF, Martinez Alonso JC, Alonso Llamazares A, et al. Fluorocytometric analysis of induced sputum cells in an asthmatic population. *J Investig Allergol Clin Immunol* 2004;**14**(2):108–13.
40. Krakowiak A, Krawczyk-Adamus P, Dudek W, Walusiak J, Palczynski C. Changes in cellular and biochemical profiles of induced sputum after allergen-induced asthmatic response: method for studying occupational allergic airway inflammation. *Int J Occup Med Environ Health* 2005;**18**(1):27–33.
41. Ahlstrom-Emanuelsson CA, Greiff L, Andersson M, Persson CG, Erjefalt JS. Eosinophil degranulation status in allergic rhinitis: observations before and during seasonal allergen exposure. *Eur Respir J* 2004;**24**(5):750–7.
42. Ferreira MB, Santos AS, Santos MC, Carlos ML, Barbosa MA, Carlos AG. Nasal ECP patterns and specific immunotherapy in mite-allergic rhinitis patients. *Allerg Immunol (Paris)* 2005;**37**(3):96–102.
43. Yu J, Yoo Y, Kim do K, Kang H, Koh YY. Bronchial responsiveness and serum eosinophil cationic protein levels in preschool children with recurrent wheezing. *Ann Allergy Asthma Immunol* 2005;**94**(6):686–92.
44. Grunberg K, Smits HH, Timmers MC, et al. Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum in asthmatic subjects. *Am J Respir Crit Care Med* 1997;**156**(2 Part 1):609–16.
45. Wilson N, Pedersen S. Inflammatory markers in clinical practice. *Am J Respir Crit Care Med* 2000;**162**(2 Part 2):S48–51.
46. Sun TY, Guo YF, Zhang HS. A study on the role of T lymphocytes and eosinophil cationic protein in exercise-induced asthma. *Zhonghua Jie He He Hu Xi Za Zhi* 2003;**26**(3):148–51.
47. Selnes A, Dotterud LK. No association between serum eosinophil cationic protein and atopic dermatitis or allergic rhinitis in an unselected population of children. *J Eur Acad Dermatol Venereol* 2005;**19**(1):61–5.
48. Vila L, Sanz ML, Sanchez-Lopez G, Garcia-Aviles C, Dieguez I. Variations of serum eosinophil cationic protein and tryptase, measured in serum and saliva, during the course of immediate allergic reactions to foods. *Allergy* 2001;**56**(6):568–72.
49. Marcucci F, Sensi L, Di Cara G, et al. Three-year follow-up of clinical and inflammation parameters in children monosensitized to mites undergoing sub-lingual immunotherapy. *Pediatr Allergy Immunol* 2005;**16**(6):519–26.
50. Badar A, Saeed W, Hussain MM, Aslam M. Correlation of eosinophil cationic protein with severity of asthma. *J Ayub Med Coll Abbottabad* 2004;**16**(3):66–71.
51. Bartoli ML, Bacci E, Carnevali S, et al. Clinical assessment of asthma severity partially corresponds to sputum eosinophilic airway inflammation. *Respir Med* 2004;**98**(2):184–93.
52. de Marco R, Marcon A, Jarvis D, Accordini S, Almar E, Bugiani M, et al. European community respiratory health survey therapy group. Prognostic factors of asthma severity: a 9-year international prospective cohort study. *J Allergy Clin Immunol* 2006;**117**(6):1249–56.
53. Jain NCR, Gleason MC, Newell Jr JD, et al. Quantitative computed tomography detects peripheral airway disease in asthmatic subjects. *Pediatr Pulmonol* 2005;**40**(3):211–8.
54. Prehn A, Seger RA, Torresani T, Molinari L, Sennhauser FH. Evaluation of a clinical algorithm involving serum eosinophil cationic protein for guiding the anti-inflammatory treatment of bronchial asthma in childhood. *Pediatr Allergy Immunol* 2000;**11**(2):87–94.
55. McCarren M, McDermott MF, Zalenski RJ, et al. Prediction of relapse within eight weeks after an acute asthma exacerbation in adults. *J Clin Epidemiol* 1998;**51**(2):107–18.
56. Wolthers OD, Heuck C. Impact of age and administration regimens on the suppressive effect of inhaled glucocorticoids on eosinophil markers in children with asthma. *Pediatric Asthma, Allergy & Immunology* 2004;**17**(1):45–51.
57. Prehn A, Seger RA, Faber J, et al. The relationship of serum-eosinophil cationic protein and eosinophil count to disease activity in children with bronchial asthma. *Pediatr Allergy Immunol* 1998;**9**(4):197–203.
58. Lonnkvist K, Hellman C, Lundahl J, Hallden G, Hedlin G. Eosinophil markers in blood, serum, and urine for monitoring the clinical course in childhood asthma: impact of budesonide treatment and withdrawal. *J Allergy Clin Immunol* 2001;**107**(5):812–7.
59. Lonnkvist K, Anderson M, Hedlin G, Svartengren M. Exhaled NO and eosinophil markers in blood, nasal lavage and sputum in children with asthma after withdrawal of budesonide. *Pediatr Allergy Immunol* 2004;**15**(4):351–8.
60. Basyigit I, Yildiz F, Kacar Ozkara S, Boyaci H, Ilgazli A, Ozkarakas O. Effects of different anti-asthmatic agents on induced sputum and eosinophil cationic protein in mild asthmatics. *Respirology* 2004;**9**(4):514–20.
61. Szefler SJ, Phillips BR, Martinez FD, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005;**115**(2):233–42.
62. Overbeek SE, O'Sullivan S, Leman K, Mulder PG, Hoogsteden HC, Prins JB. Effect of montelukast compared with inhaled fluticasone on airway inflammation. *Clin Exp Allergy* 2004;**34**(9):1388–94.
63. Kawano T, Matsuse H, Kondo Y, et al. Tacrolimus reduces urinary excretion of leukotriene E(4) and inhibits aspirin-induced asthma to threshold dose of aspirin. *J Allergy Clin Immunol* 2004;**114**(6):1278–81.
64. Kandil AA, Hasan A, Taha O, El-Mesallamy H. Eosinophil cationic protein as a diagnostic marker for asthmatic children treated by immunotherapy. *Egypt J Immunol* 2003;**10**(1):67–76.
65. Fardon TC, Lee DK, Hodge MR, Lipworth BJ. Addition of fexofenadine to inhaled corticosteroid therapy to reduce inflammatory biomarkers in atopic asthma. *Ann Allergy Asthma Immunol* 2005;**95**(3):259–65.
66. Koopmans JG, Lutter R, Jansen HM, van der Zee JS. Adding salmeterol to an inhaled corticosteroid reduces allergen-induced serum IL-5 and peripheral blood eosinophils. *J Allergy Clin Immunol* 2005;**116**(5):1007–13.
67. King MJ, Bukantz SC, Phillips S, Mohapatra SS, Tamulis T, Lockey RF. Serum total IgE and specific IgE to Dermatophagoides pteronyssinus, but not eosinophil cationic protein, are more likely to be elevated in elderly asthmatic patients. *Allergy Asthma Proc* 2004;**25**(5):321–5.
68. Pedersen B, Dahl R, Karlstrom R, Peterson CG, Venge P. Eosinophil and neutrophil activity in asthma in a one-year trial with inhaled budesonide. The impact of smoking. *Am J Respir Crit Care Med* 1996;**153**(5):1519–29.
69. Wolthers OD, Heuck C. Circadian variations in serum eosinophil cationic protein, and serum and urine eosinophil protein X. *Pediatr Allergy Immunol* 2003;**14**(2):130–3.

70. Ferdousi HA, Munir AK, Zetterstrom O, Dreborg SK. Seasonal differences of peak expiratory flow rate variability and mediators of allergic inflammation in non-atopic adolescents. *Pediatr Allergy Immunol* 2001;**12**(5):238–46.
71. Zietkowski Z, Bodzenta-Lukaszyk A, Tomasiak MM, Skiepko R, Szmitkowski M. Comparison of exhaled nitric oxide measurement with conventional tests in steroid-naive asthma patients. *J Invest Allergol Clin Immunol* 2006;**16**(4):239–46.
72. Wennergren G. Inflammatory mediators in blood and urine. *Paediatr Respir Rev* 2000;**1**(3):259–65.
73. Baraldi E, Carraro S. Exhaled NO and breath condensate. *Paediatr Respir Rev* 2006;**7**(1):S20–2.
74. Zhou QT, Sun YC, Yao WZ. Characteristics of the airway inflammation and the relationship to interleukin-17 in severe asthma. *Zhonghua Jie He He Hu Xi Za Zhi* 2005;**28**(9):630–4.